AMERICAN PETROLEUM INSTITUTE PROJECT 43 AND THE ORIGIN OF MODERN PETROLEUM MICROBIOLOGY

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ABSTRACT: In 1942, American Petroleum Institute Project 43 was initiated with the assistance of three major university research groups and was funded via private industry. Fueled by the WWII war efforts, the aims of the project were to investigate the roles of physical, chemical and biological forces in the transformation of organic material into petroleum. Basic studies on microbial activities related to petroleum recovery and degradation were directed by marine microbiologist Dr. Claude ZoBell at Scripps Institution of Oceanography and the results were published extensively in journals and oil industry publications between 1943 and 1955. Studies by ZoBell's group established the presence of bacteria in recent and ancient sediments, in oil field fluids recovered from great depths, and living at extremely high temperatures and pressures. Specific bacteria were found to be instrumental in liberating oil from oil-bearing sedimentary rocks by dissolving carbonates, producing detergents, and decreasing the viscosity of oil. In addition, the ability of bacteria to degrade hydrocarbons was shown to be relatively commonplace, a characteristic that has been widely exploited in the bioremediation of accidental oil spills.

INTRODUCTION

Microbial activity is known today to have significant positive and negative impacts on the petroleum industry. From a beneficial perspective, microscopic bacteria and fungi have been used to prospect for oil, recover oil from subsurface reservoirs, biosynthesize edible proteins, and remediate petroleum-contaminated soil and water (Hamer and Al-Awadhi 2000). Detrimental activities include the microbial corrosion of iron and steel, the plugging of reservoirs, the degradation of drilling fluid additives, the contamination of fuels, and the deterioration of asphalts (Davis 1967). The wide variety of these microbial activities and the extent of our current knowledge about them belies the fact that most of this information has only been discovered in the last 50 years. The compound microscope was invented near the end of the 16th century, and the first microorganisms were observed and described by van Leeuwenhoek in 1675, but microbiology was still many years from becoming a legitimate field of science. Its development, in fact, paralleled that of the infant petroleum industry. Louis Pasteur, often considered to be the "father of microbiology," was not even born until 1822. His scientific proof that bacteria did not just appear miraculously by the process of spontaneous generation was published in 1860, the year after the drilling of Drake's successful oil well in Titusville, PA (Beerstecher 1954). By the time of World War I, isolated academic studies had demonstrated that bacteria could produce methane from cellulose and decompose selected aliphatic and aromatic hydrocarbons, but few practical microbiological applications had been developed that might be considered useful to the petroleum industry.

THE AMERICAN PETROLEUM INSTITUTE

In 1925, John D. Rockefeller Sr. donated $250,000 to help establish the American Petroleum Institute (API), which was to support fundamental research related to the occurrence, discovery, production and refining of petroleum (Davis 1967). Additional contributions from petroleum companies led to the creation of an API research program that was largely supported by industry. Beginning in 1926, the API, along with the United States Geological Survey (USGS) and the National Research Council, sponsored a long-term research project entitled the "Origin and Environment of Source Sediments of Petroleum" (Knebel 1946). The principal objectives of this study were to look for evidence that would indicate why certain recent sediments might ultimately become source sediments of petroleum. API Project 4, as it came to be known, was directed by Parker D. Trask, a research associate with the API and the USGS. Trask and his co-workers collected more than 35,000 samples of recent and ancient marine sediments from all over the world (Beerstecher 1954). The funding for this work continued through 1941, and resulted in two significant publications related to the source sediments of petroleum (Trask 1932; 1942). However, even though the results of this work strongly suggested that most petroleum originated from organic matter in marine sediments, definitive proof of the mechanisms of petroleum formation and its sources could not be determined. What petroleum geologists still needed in their quest for oil were scientific criteria that would allow them to differentiate between sediments that had either produced or not produced petroleum. The problem was stated succinctly by Heald (1955):

> "If it can be known how oil is formed and if the formation in which it was generated can be recognized and classified as rich, average, or poor, the search for new fields would be greatly simplified; the efforts spent on areas where oil will not be found would be greatly reduced; and the search would be intensified in areas where, despite early failure, oil should exist."

SCIENTIFIC COLLABORATION AND THE "GEOLOGICAL FENCE"

At this point in history, a rather refreshing collaboration between the petroleum industry and the science of microbiology occurred. Geologists knew that plant and animal remains were found in sediments, and that petroleum was likely to originate from this material. Since major chemical transformations would clearly be required to transform these materials into petroleum, the potential role of either bacteria or radioactive minerals in sediments suggested themselves as potential catalysts of the process. Geologists also knew that specialists in these two fields would need to be enlisted from academia to carry out meaningful research on the topic.

To guide the direction of any new research and bring potential collaborators up-to-date on the existing geological evidence and known physical and chemical constraints on petroleum formation, these data were summarized in April 1941 in a statement that later became known as the "Geological Fence." This "fence" was intended to describe the boundaries that would constrain the new research activities by what were considered by geologists to be irrefutable "fence posts": A) that petroleum was found in sediments of marine origin; B) that temperature and pressure in oil fields fell within certain known boundaries; and C) that no geological evidence existed proving that any petroleum had been formed within the past 1 million years (Cox 1946). Other observations ("stakes" in the fence) covered points regarding what was known about radioactivity, bacteria, catalysts, variations in composition and properties of petroleum, and hydrocarbons formed by living plants (Knebel 1946).

Six proposals designed to tackle specific aspects of the problem were submitted by various universities in the summer of 1941. Of these, 3 projects were eventually chosen, streamlined, and finally accepted by the API in the spring of 1942. Projects A, B and C were designed to collaborate with one another under the heading of Project 43. These included, respectively, "The Role of Naturally Occurring Biochemical Agents in Petroleum Genesis," directed by Dr. Claude E. ZoBell of Scripps Institution of Oceanography (SIO), La Jolla, CA; "Studies in the Fields of Chemistry, Bacteriology, Physical Chemistry, and Physics," directed by Dean Frank C. Whitmore, Pennsylvania State University (PSU), State College, PA; and "Studies of the Effect of Radioactivity on the Transformation of Marine Organic Material into Petroleum Hydrocarbons," directed by Prof. Warren J. Meade, Massachusetts Institute of Technology (MIT), Cambridge, MA. Although all three projects contributed to the list of the accomplishments of Project 43 in subsequent years, I will principally discuss the microbiological studies of Dr. ZoBell and his colleagues at SIO in the remainder of this paper.

CLAUDE E. ZOBELL

Claude ZoBell (Fig. 1) was born in Provo, Utah, and like most scientists in the relatively new discipline of what would become known as "environmental microbiology," he had a strong background in medical microbiology. He received his Ph.D. from the Hooper Foundation for Medical Research at the University of California in 1931 after a two-year stint as the principal of a public school in Utah. He eventually settled on a position at SIO and pursued his scientific curiosity about bacteria living in the sea. He became the first marine microbiologist to examine all parts of the ocean environment systematically for signs of microbial life (Ehrlich 1999). He essentially demonstrated that bacteria were found throughout the ocean environment without regard for extremes in temperature, pressure and salinity (ZoBell 1946b), and developed novel (Fig. 2a) and sometimes unusual (Fig. 2b) methods for sampling the marine environment. The presentation of data at professional geological conferences in the late 1930s, suggesting that bacteria were ubiquitous in the same marine environments believed to ultimately be the source of petroleum, brought Dr. ZoBell's work to the attention of petroleum geologists and the API (Knebel 1946; Bass 1999).

The scope of work proposed to the API by the SIO group was both broad and ambitious. API Project 43A would:

1. Investigate the role of marine bacteria in the transformation of organic material into petroleum;

2. Study the occurrence of hydrocarbons in ocean-bottom sediments;

3. Obtain cores of ocean-bottom sediments for study;

4. Supply samples of ocean-bottom sediments to co-workers at Penn State and MIT;

5. Search for and study bacteria found in oil-well brines, crude oils, rock cores, and other geological sources;

6. Identify the chemical nature of tarry and oily material separated from marine muds;

Figure 1. Claude E. ZoBell (Beerstecher, 1954).

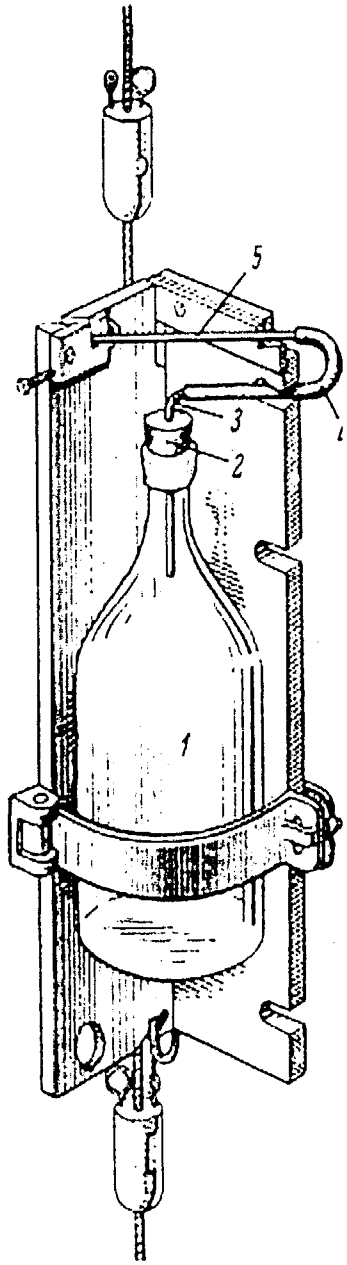

Figure 2A. ZoBell's J-Z bacteriological water sampler (Rodina 1972; after ZoBell 1941).

7. Identify the chemical nature of the above and study its alteration by marine bacteria;

8. Isolate and identify marine bacteria and study their ability to transform known organic compounds;

9. Study the effects of marine bacteria on the hydrogen ion concentration (pH) and the oxidation reduction potential (Eh) of marine bottom sediments; and

10. Develop methods to determine the Eh of petroliferous materials.

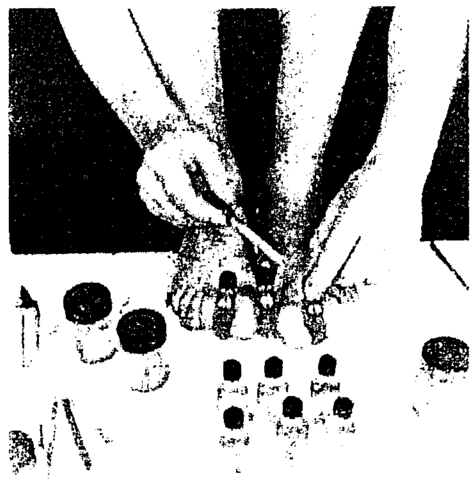

Figure 2B. Dr. ZoBell inoculating microbiological media with sediment samples during the Galathea Deep Sea Expedition, 1950-1952 (Bruun et al., 1956).

Clearly, completing this work would be no easy task. In retrospect, the initial proposal also demonstrates the savvy of a seasoned scientist who had to satisfy the demands of the API and other research granting agencies, in that many of these tasks were presumably straight forward extensions of work ZoBell had already pioneered. Items 1,2, 8, and 9 had been areas of research in his lab during the 1930s and would be well-described by the time he published the first textbook on marine microbiology a few years later (ZoBell 1946b). The availability of SIO's research vessel, the *E. W. Scripps*, should allow the accomplishment of items 3 and 4 with relative ease (Fig. 3). Oil companies had agreed to send core samples and provide access to unique sites, such as the oil "mine" near Franklin, PA, currently under construction (Fig. 4). Additionally, these objectives realistically allowed for the continuation of research for as long as the granting agency was happy with the progress. Underlying this apparent aura of self-preservation, however, was the reality that all of Project 43 received an initial annual budget of only $17,800, all privately subscribed from industry (Knebel 1946).

The SIO group was also expected to collaborate with the other two Project 43 subgroups, especially with Project 43B at PSU, which had a number of microbiological tasks to perform. They were to:

1. Study the enzymatic action of bacteria isolated from petroleum and from sediments on the degradation of proteins, carbohydrates, fats, hydrocarbons, and other organic compounds;

2. Study known and new species of bacteria for their ability to produce hydrocarbons;

3. Study the action of bacteria on petroleum and its constituents; and

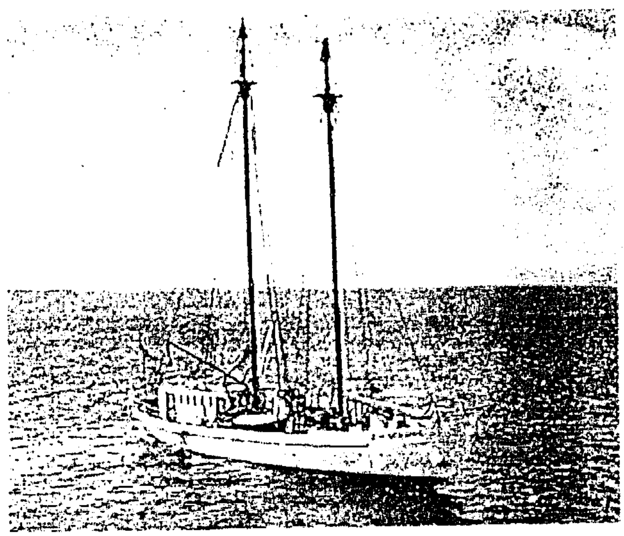

Figure 3. The E.W. Scripps, the 104-ft research vessel of the Scripps Institution of Oceanography used by the Project 43a team (ZoBell 1950).

Figure 4. Construction of the "oil mine" near Franklin, PA in August 1942. The shaft was 8 ft in diameter and was sunk ca. 430 ft to the Venango First Sand (Courtesy of the Drake Well Museum).

4. Attempt to isolate bacteria from uncontaminated petroleum, study the characteristics of any such bacteria, and identify the species (Knebel 1946).

SIO would provide the marine sediment cores that would be the source of raw material for these studies, and the nearby oil fields of Pennsylvania would provide convenient and accessible field sites for the collection of samples of petroleum and rock samples from petroleum formations. This, at least, was the plan.

WAR-RELATED PROBLEMS

Given a choice of time in history in which to start an ambitious ocean-based research project, it is unlikely that one would deliberately choose the year 1942. Granted, much of the push to investigate petroleum sources and exploration methods was driven by the need to support the growing military requirement for oil, but there were many negative side-effects on the research project as well. Firstly, SIO's research vessel was taken over by the U.S. Navy for the duration of the war, and scientists were forced to use sediment cores collected during pre-War years. Lab personnel (Fig. 5) were continuously being drafted for the war and materials were in short supply. At PSU, principal investigators were redirected almost entirely towards important war-related efforts, such as penicillin research. However, despite these problems, considerable work was accomplished, especially by Project 43A at SIO.

PROJECT 43 ACCOMPLISHMENTS

Rather than provide a year by year account of the scientific progress of Project 43, I will jump ahead to its eventual termination in 1953 and summarize its major accomplishments and disappointments. The reader interested in the historical development of this research is directed to the six annual reports of progress (American Petroleum Institute 1944, 1946, 1949, 1950, 1952, 1955) available in some major libraries, as well as the overall summation of Project 43 by Heald in the final report (1955).

From the perspective of the petroleum industry, Project 43 went a long way towards disproving the notion that petroleum was produced directly by microorganisms. As summarized by Heald (1955):

> "The results from a number of lines of investigation would have to be classed as inconclusive at the time the support of the American Petroleum Institute was terminated, but none of these incomplete studies promised to change the significant conclusion that bacteria unaided cannot transform naturally occurring organic matter into petroleum."

Figure 5. Team members of API Research Project 43a ca. 1946. L to R: W.D. Rosenfeld, D.M. Updegraff, H.H. Whelpy, F.D. Sisler, C.E. ZoBell, and W.E. Hutton (ZoBell, 1947a).

PSU researchers developed methods to grow up to 10 kg quantities of marine bacteria initially supplied by Dr. ZoBell's group, but could only show that relatively small amounts of petroleum-like hydrocarbons were present in the bacterial biomass. There was no doubt that bacterial activity in recent marine sediments was responsible for some degree of organic matter degradation and transformation - but how this degraded material was converted to petroleum remained a mystery.

> "If we accept the geologists' conclusion that organic material is the mother substance of petroleum, we add as a corollary to this premise that microorganisms must have contributed to the process of formation. The soil and marine microbiologist expects to find evidence of extensive and complex microbial activity wherever he finds organic matter deposited, whether it be in fresh or salt water, cold or warm temperatures, aerobic or anaerobic atmosphere and, in moderation, high pressure or low. From the standpoint of the microbiologist as well as the chemist and geologist, the critical question appears to be "how far along the road to crude oil can microorganisms conduct the raw organic material?" Stone and ZoBell (1952)

At SIO, Dr. ZoBell's group made numerous findings, some of which seem almost simplistic today, but which were extremely important in the context of their discovery in the 1940s. Among these, Project 43A found that:

1. Biochemically diverse bacteria were found in both ancient and recent marine sediments. Bacteria isolated from a Louisiana salt dome cap by drilling were reasonably certain to have been indigenous to the formation, suggesting long-term survival in ancient sediments;

2. Great numbers of bacteria were found in oil well fluids obtained from depths of several thousand feet;

3. Bacteria could grow at temperatures ranging from 0 to 85°C and at hydrostatic pressures of up to 150,000 psi;

4. Bacteria were shown to be influential in creating an oxygen-free environment in marine sediments, protecting buried organic matter from complete oxidation by aerobic microorganisms;

5. Bacteria were found to be capable of releasing oil from oil-bearing sedimentary rocks by dissolving carbonates, producing detergents, forcing out oil during growth, or breaking down long-chain hydrocarbons, raising the potential for microbial enhanced oil recovery (MEOR);

6. Certain crude oils were found to inhibit the growth of bacteria due to their heavy metal content; and

7. Aerobic bacteria capable of degrading hydrocarbons were abundant in nature.

Finding #5 was of special interest to the oil industry. ZoBell demonstrated that the addition of sulfate-reducing bacteria to Athabasca oil sands appeared to stimulate the release of oil (Novelli and ZoBell 1944; ZoBell 1946a, 1947a, 1947b; Fig. 6). Numerous experiments were conducted almost immediately at the Pennsylvania Grade Crude Oil Association laboratory in Bradford, PA, with the assistance of ZoBell as a consultant. However, the initial results were mixed, and the research was discontinued for lack of time and funds (Beck 1947).

In addition, many other ancillary discoveries and techniques of interest to the scientific community were made, such as the identification of the many bacteria that were isolated, the development of sampling techniques, etc. However, these benefits were of lesser interest to the petroleum industry, as stated rather bluntly by Heald (1955):

> "Individuals and organizations, such as universities, museums, and government bureaus, may devote time and money to studies designed exclusively to increase the sum of human knowledge, but this is not permissible for industry."

Nevertheless, Project 43 convinced the petroleum industry that microbiology was a science to be reckoned with, if not exploited. Major oil companies established microbiology projects in many of their own research labs and successfully lured many of the leading scientists in the field away from academia (Sharpley 1966). Other academicians turned their attention to research issues related to oil production and the microbial contamination of fuels in the transportation industry (Sharpley 1966). Ocean drilling brought with it the contamination of wells with sulfate in seawater and the "souring" of the wells, or the production of hydrogen sulfide by anaerobic bacteria, with costly metal corrosion and human health hazards (Davis 1967). The development of the supertanker for long distance oil transportation in the 1960s led to public recognition of the potential for massive releases of petroleum in the ocean and subsequent environmental damage

Figure 6. Microbial release of oil from Athabasca tar sand. Experiments demonstrated the ability of sulfate-reducing bacteria to free oil from tar sands (bottles B and C) relative to uninoculated controls (bottles A and D; ZoBell, 1947b).

(Atlas 1981). An examination of the rate of publication of journal articles dealing with petroleum microbiology prior to 1966 illustrates that real interest in petroleum microbiology as a science coincides with the groundbreaking research of Project 43 (Fig 7). It was estimated that fully one-sixth of all published papers on the subject was produced by ZoBell's group during the period 1943 to 1953 (Beerstecher 1954).

CONCLUSIONS

Despite the limitations imposed by a World War, Project 43 was successful in several respects. It firmly established the fact that bacteria were ubiquitous globally, had the widespread ability to degrade hydrocarbons, and were of potential utility to the petroleum industry. The technique of microbially-enhanced oil recovery, a tertiary recovery method first explored by ZoBell during this project, may become a technology of the future, as the great quantities of unextracted oil remaining in old reservoirs look more and more attractive to petroleum geologists and the general public. The ability of microorganisms to degrade hydrocarbons continues to be exploited as an important tool in the remediation of oil-contaminated waters and soils.

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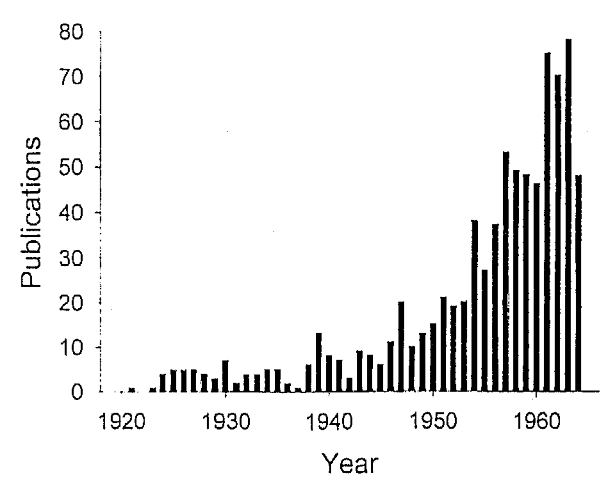

Figure 7. Publication rate of petroleum microbiology-related journal articles by year, based on 838 total references cited in the 1967 textbook "Petroleum Microbiology" (Davis 1967).

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